1140-Plat
Conformational Selection and Allosteric Regulation upon Ligand Binding in G-Protein Coupled Receptors
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G-protein coupled receptors (GPCRs) are allosteric membrane proteins mediating cellular signaling. GPCRs exhibit multiple inactive and active conformations, and the population balance between these conformations is altered upon binding of signaling molecules (or ligands). However, the nature of the conformational ensemble or the mechanism of the conformational transitions is not well understood. We have applied a multiscale computational approach combining a coarse-grained discrete conformational sampling method with fine-grained molecular dynamics to investigate the effect of various ligands binding on the ensemble of conformations sampled by human β2-adrenergic receptor (β2AR). We show that the receptor, in the absence of any ligand, samples an extensive conformational space that includes breathing of the orthosteric ligand binding site and shear motion of the transmembrane helices 5 and 6 against the other helices. The shear motion is similar to the reorganization of the intracellular regions of TM3, TM5, and TM6 observed in the crystal structure of the active state of GPCRs. Upon ligand binding a shift in population density, as well as a reduction in the number of conformations sampled by the receptor is observed. Binding of agonist norepinephrine or partial agonist salbutamol leads to the selection of a subset of conformations that include both active and inactive state conformations, while inverse agonist carazolol selects only the inactive state conformation. Possible mechanisms for the allosteric regulation of GPCR activity by ligand binding were identified by correlating receptor-ligand interactions with receptor sidechain orientation near the G-Protein coupling region.

1141-Plat
Refining Protein Interaction Networks with Protein Structure and Kinetic Modeling
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Protein interaction networks build off both high-throughput and curated experimental data to provide a global picture of the complex and interconnected functional pathways in the cell. However, these networks represent only a time averaged snapshot of the dynamic cellular environment where it is difficult to assess the strength, frequency, and uniqueness of pairs of binding partners. We demonstrate through computational modeling and network analysis how a protein's position in a network and the overall topology of the network can influence the number of interfaces the protein uses, its binding equilibrium, and its concentration. Our systematic analysis provides a practical tool for improving the details presented in a protein interaction network and therefore better characterizing the dynamics of the protein interactions. This analysis also presents a means for predicting false negatives in small networks. Our model of protein interactions explicitly accounts for the ability of proteins to form nonspecific complexes through transient encounters in the crowded cellular environment. This work further supports the hypothesis that the need to suppress nonfunctional interactions acts as a control on the types of protein interaction networks and the relative concentration of proteins observed in cellular compartments.

Platform: Calcium Fluxes, Sparks, and Waves

1142-Plat
Inositol 1,4,5 Trisphosphate Receptor Type 1 (IP_3R1) Activate Ryanodine Receptor (RyR1) to Mediate Ca^{2+} Spark Signaling in Adult Mammalian Skeletal Muscle
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The functional coupling between IP_3R and RyR1 in skeletal muscle have not been extensively studied. Ca^{2+} sparks, a RyR1-dependent Ca^{2+} release events are rarely observed during resting condition in adult mammalian skeletal muscle due to the inhibitory function of the EC-coupling machinery. Previously we showed that membrane deformation elicited by osmotic stress generated robust Ca^{2+} sparks that are restricted to the periphery of sarcolemma in skeletal muscle. Here we tested the hypothesis that lipid signaling mediated by membrane deformation activates Ca^{2+} release from IP_3R that subsequently leads to activation of neighboring RyR, producing robust and localized Ca^{2+} spark events. Immunohistochemical staining showed that IP_3R1 and IP_3R2 were localized along the subsarcolemma region, coinciding with the distribution of Ca^{2+} sparks in skeletal muscle. It is known that transient exposure of cells to osmotic stress could elevate $PI(4,5)P_2$ and IP_3 levels inside the cell. Pharmacological interventions blocking the activation of IP_3R suppress the osmotic-stress induced Ca^{2+} spark events in skeletal muscle. Using the $IP_3R2^{-/-}$ mice, we showed that stress-induced Ca^{2+} sparks was not affected, suggesting that the type 2 IP_3R is not involved in the onset of Ca^{2+} sparks in skeletal muscle. Specific silencing of the type 1 IP_3R from the wild type or the $IP_3R2^{-/-}$ mice led to complete ablation of the stress-induced Ca^{2+} sparks in skeletal muscle. Overall, our findings reveal a functional coupling between $IP_3R1$and RyR1 in regulating intracellular Ca^{2+} signaling in skeletal muscle. The stress induced Ca^{2+} sparks likely involve a cross-talk between IP_3R1 and RyR1, which must overcome the inhibitory action of the EC coupling machinery at resting condition that is essential for the tight control of muscle contraction.

1143-Plat
Role of Cytoplasmic Buffers in Spatial H^+-Ca^{2+} Interactions in Ventricular Myocytes
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Cytoplasmic Ca^{2+} is modulated by H^+-ions. We investigated the relationship between intracellular pH (pH_i) and diastolic or resting Ca^{2+} ($[Ca^{2+}]_{dia}$) in confocally-imaged rat myocytes loaded with Fluo-3 (Ca^{2+}) or cSNARF-1 (pH_i). Intracellular release of H^+-ions from a membrane-permeant weak-acid (80mM acetate) or photo-labile caged H^+-compound (2-nitrobenzaldehyde) produced a rise in $[Ca^{2+}]_{dia}$. This rise was restricted spatially to the acid-microdomain formed by H^+-release. The resulting cytoplasmic Ca^{2+}-gradient was maintained for the lifetime of the underlying pH_i-microdomain, but could be dissipated by AM-loaded BAPTA (a Ca^{2+}-buffer). The spatial H^+-Ca^{2+} interaction was not abolished by removal of extracellular Na^+ or Ca^{2+}, or by exposure to thapsigargin (10μM), caffeine (10mM), bafilomycin (5μM), glycyl-L-phenylalanine 2-naphthylamide (100μM) or ruthenium-360 (10μM). However, inhibition of mitochondrial respiration with FCCP (1-5μM), rotenone (10μM), myxothiazole (10μM), or of glycolysis with deoxyglucose (2mM), reduced the H^+-evoked $[Ca^{2+}]_{dia}$-rise in a manner that correlated with the decline of [ATP] (luciferase assay). We conclude that the H^+-evoked Ca^{2+}-rise is not due to extracellular Ca^{2+}-influx, or Ca^{2+}-release from organelles. It most likely arises from competitive Ca^{2+}/H^+-binding to cytoplasmic buffers, some of which are diffusively mobile and therefore engage in spatial Ca^{2+}/H^+ exchange, resulting in recruitment of Ca^{2+} to acidic microdomains. We therefore investigated the *in vitro* pH-sensitivity of Ca^{2+}-binding to a range of known cytoplasmic buffers, using H^+-uncaging in agar-set mixtures. We find that histidyl-dipeptides (e.g. carnosine) and ATP are the myocyte's principal pH-sensitive mobile Ca^{2+}-buffers. Involvement of Ca^{2+}-bound ATP confers metabolic sensitivity to the cytoplasmic H^+-Ca^{2+} interaction. Results indicate that cytoplasmic pH_i-microdomains, formed during membrane H^+- or weak-acid transport will generate Ca^{2+}-microdomains. These may help to compensate for the reactive effects of H^+-ions on Ca^{2+}-dependent protein function. *Supported by the British Heart Foundation, NIH and Royal Society.*

1144-Plat
Calcium Handling Derangement is Associated with Conditional Cardiac Myosin Binding Protein C Knock Out
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The majority of sudden cardiac arrest (SCA) in young people is attributable to heritable cardiomyopathy (HCM), a relatively common disease affecting 1 in 500 individuals. The preponderance of HCM-associated mutations has been identified in sarcomeric proteins, particularly β-myosin heavy chain and cardiac myosin binding protein-C (cMyBP-C, encoded by MYBPC3). cMyBP-C, an accessory protein that binds tightly to myosin, has an important role in thick filament regulation. Explanted hearts from MYBPC3-null mice exhibit greater susceptibility to arrhythmias than wild-type (WT), which is suggestive of Ca^{2+} dysregulation. We investigated this possibility with tamoxifen-induced conditional MYBPC3 knock out mice (cKO), which enabled us to examine possible molecular mechanisms of the disease in the relative absence of hypertrophic remodeling. First, the propensity for spontaneous Ca^{2+} release was greater in cKO-CMs than in WT-CMs, suggesting alterations in intracellular Ca^{2+} handling. In line with this notion, cKO-CMs displayed dramatic prolongation of the time constant (τ) of transient decay (mean 217 ± 18 msec for cKO-CMs vs. 107 ± 4 msec in WT CMs) and increased diastolic signal. Additionally, Western blots revealed downregulation of NCX, SERCA, and pentameric PLN–the net effect of which would be to slow the resequestration of Ca^{2+} into the sarcoplasmic reticulum while preventing depletion of SR Ca^{2+} stores. Further, as a consequence of the $[Ca^{2+}]_i$ alterations or secondary to MYBPC3 knockout, cell shortenings were impaired in cKO-CMs (reduced by as much as 54% versus WT-CMs), while amplitude was significantly depressed (over 20% lower than WT-CMs). Lastly, Langendorff perfused cKO hearts exhibited electrical instability, which was not observed in WT hearts. These maladaptive alterations, as demonstrated in our single cell